Dissipation of Antimicrobials in a Seasonally Frozen Soil after Beef Cattle Manure Application

Inoka D. Amarakoon, Srinivas Sura, Francis Zvomuya,* Allan J. Cessna, Francis J. Larney, and Tim A. McAllister

Abstract

Land application of manure containing antimicrobials results in the dispersion of the antimicrobials in agro-ecosystems. Dissipation of excreted antimicrobials in seasonally frozen agricultural soils has not been fully characterized under field conditions. This study investigated the field dissipation kinetics of chlortetracycline, sulfamethazine, and tylosin over a 10-mo period after fall application of manure from cattle (Bos taurus) administered 44 mg chlortetracycline (chlortetracycline treatment (CTC)), 44 mg each of chlortetracycline and sulfamethazine (CTCSMZ), or 11 mg tylosin per kg feed daily. Antimicrobial concentrations in manured soil reflected the same relative concentrations in manure: chlortetracycline > sulfamethazine > tylosin. The first-order dissipation half-life (DT50) for chlortetracycline from the CTCSMZ treatment was 77 d during the growing season and 648 d during the nongrowing season when the soil was frozen for an extended period. By comparison, dissipation of chlortetracycline added alone (treatment CTC) did not differ significantly between the two seasons (mean DT50 = 121 d). During the nongrowing season, chlortetracycline from CTC dissipated faster (P = 0.004) than that from the CTCSMZ treatment, indicating that the presence of sulfamethazine may have altered the dissipation of chlortetracycline. Dissipation kinetics for sulfamethazine and tylosin were not determined due to low detection in the manure-amended soil. Sulfamethazine was detected (up to 16 ± 10 µg kg−1) throughout the 10-mo monitoring period. Tylosin concentration was ≤ 11 ± 6.6 µg kg−1 and gradually dissipated. Chlortetracycline was detectable 10 mo after application in the seasonally frozen soil, indicating a risk for residue build-up in the soil and subsequent offsite contamination.

Core Ideas

- Manure application can disperse antimicrobials in the environment.
- Chlortetracycline dissipation followed first-order kinetics.
- Seasonal effects on chlortetracycline dissipation varied with the source of the antimicrobial.
- The presence of sulfamethazine slowed down the dissipation of chlortetracycline.
- Concentrations of tylosin and sulfamethazine in manured soil were low.

Abbreviations: CTC, chlortetracycline treatment; CTCSMZ, treatment consisting of a 1:1 mixture of chlortetracycline and sulfamethazine; DT50, time for 50% of initial antimicrobial concentration to dissipate; LC/MS/MS, liquid chromatography-tandem mass spectrometry; MAE, microwave-assisted extraction; TYL, tylosin treatment.

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needed to understand the environmental fate and, potentially, environmental mobility of these compounds. However, research on the persistence of these antimicrobials in the environment is still an emerging area, with only a few studies reported to date.

A number of laboratory microcosm studies using fortified soils have been conducted to examine the dissipation of these antimicrobials in soils. Topp et al. (2013) reported time for 50% of initial antimicrobial concentration to dissipate \((DT_{50})\) values of 3.3 and 2.8 d for chlortetracycline, 1.3 and 5.3 d for sulfamethazine, and 2 and 10 d for tylosin when soils collected from field plots in Ontario, Canada, were fortified with these antimicrobials. Sassman et al. (2007) observed \(DT_{50}\) values of 23 to 24 d for tylosin in surface soils collected from six midwestern United States and in one sandy soil collected from Florida. Accinelli et al. (2007) reported a \(DT_{50}\) of 18.6 d for sulfamethazine in silty loam and sandy soils collected from sites in Minnesota. Schlesener and Bester (2006) reported a \(DT_{50}\) of 8 d for tylosin in a sandy loam surface soil collected from Germany. Although such laboratory microcosm studies have merit, they are often performed in controlled environments that are optimal for antimicrobial degradation, conditions that may not be representative of the more complex dissipation processes that occur under variable field conditions.

Chlortetracycline concentrations in farmlands in Germany were 5 to 7 \(\mu g\) kg\(^{-1}\) soil after two annual applications of liquid swine manure (Hamscher et al., 2002). When the swine manure was applied annually for 4 yr, chlortetracycline concentrations of 4 to 39 \(\mu g\) kg\(^{-1}\) were reported (Hamscher et al., 2005). Chlortetracycline residues have also been reported in Austrian farmland soils amended with swine manure (Martinez-Carballo et al., 2007). In Denmark, \(DT_{50}\) values for chlortetracycline were 25 d in a sandy loam and 34 d in a sandy soil in the summer after field application of liquid swine manure (Halling-Sorensen et al., 2005). In the same study, \(DT_{50}\) values for tylosin were 67 d in the sandy loam and 49 d in the sandy soil. In Switzerland, sulfamethazine concentration was about one half \((250–400 \mu g\) kg\(^{-1}\) wet soil\) the initial concentration \((500–700 \mu g\) kg\(^{-1}\) within 42 d of application in a soil amended with liquid swine manure (Stoob et al., 2006). In Ontario, Canada, Carlson and Mabury (2006) reported first-order \(DT_{50}\) values of 21 d for chlortetracycline and 6.1 d for tylosin during a 50-d field study in the summer after field application \((incorporation)\) of the antimicrobials mixed with sand. In the same study, half-lives of 24 d for chlortetracycline and 4.5 d for tylosin were observed in plots receiving dairy manure after antimicrobial incorporation.

To date, the few field studies that have evaluated antimicrobial dissipation in manure-amended soils have been conducted during summer months. Furthermore, none of the studies have monitored antimicrobial dissipation for a year or longer to capture seasonal effects \(e.g.,\) wet-dry and freeze-thaw cycles. There is also a dearth of published studies using manure containing antimicrobials that were orally administered to livestock and excreted, which better represents actual conditions by which antimicrobials enter agricultural soils (Amarakoon et al., 2016), as opposed to directly fortifying soil or manure with antimicrobials. Moreover, there are fewer published antimicrobial dissipation studies using cattle manure compared with those using liquid swine manure, despite a large cattle industry in North America. This study, therefore, was conducted to quantify the rates of dissipation of chlortetracycline, sulfamethazine, and tylosin in a seasonally frozen agricultural soil after fall application of beef cattle manure containing the excrated antimicrobials.

**Materials and Methods**

**Site Description**

The 2-yr experiment was conducted in 2010–2011 and repeated in adjacent plots in 2011–2012 at the Agriculture and Agri-Food Canada Research and Development Centre in Lethbridge, Alberta, Canada \(\left(49^\circ\ 42’\ N, 112^\circ\ 50’\ W\right)\). The soil was a semiarid Dark Brown Chernozem \(\left(Soil\ Classification\ Working\ Group,\ 1998\right)\) with a clay-loam texture. Organic carbon content was 15 g kg\(^{-1}\), and the pH was 7.5 at the 0- to 15-cm depth. The site had no history of manure application and had been continuously cropped to spring wheat \(\left(Triticum\ aestivum\ L.\right)\) before the experiment. The 30-yr \(\left(1981–2010\right)\) mean annual air temperature was 6.4°C, and mean annual precipitation was 399 mm \(\left(\text{as recorded at a weather station located ~}500\text{ m from the study site}\right)\).

**Antimicrobial Treatments Administered to Cattle**

Antimicrobials were administered via feed at concentrations of 44 mg kg\(^{-1}\) feed chlortetracycline \(\left(\text{chlortetracycline treatment \(\left[CTC\right]\)}\right), 44 mg kg\(^{-1}\) feed each of chlortetracycline and sulfamethazine \(\left(\text{CTCSMZ}\right)\), and 11 mg kg\(^{-1}\) feed tylosin \(\left(\text{TYL}\right)\) to nine steers housed in a pen specific to each antimicrobial treatment \(\left(Amarakoon\ et\ al.,\ 2014\right)\). A fourth pen of nine steers was used as a control where no antimicrobials were administered in the diet. In the first year \(\left(2010\right)\), the feeding period was 111 d \(\left(18\text{ June–7 October}\right)\) for all treatments. In the second year \(\left(2011\right)\), the feeding periods were 105 d \(\left(28\text{ June–11 October}\right)\) for TYL and the control and 98 d \(\left(5\text{ July–11 October}\right)\) for CTC and CTCSMZ. A manure/bedding ratio of ~4:1 was maintained in the pens by addition of barley \(\left(Hordeum\ vulgare\ L.\right)\) straw at regular intervals. Manure was allowed to accumulate throughout the feeding periods.

**Plot Set-up and Manure Application**

The experimental design was a randomized complete block with each of the four blocks \(\left(\text{replications}\right)\) containing the four antimicrobial treatments \(\left(\text{CTC, CTCSMZ, TYL, and control, as described above}\right)\). Each block was divided into four 5-m by 3-m plots \(\left(i.e.,\ one\ for\ each\ antimicrobial\ treatment\right)\).

Manure was collected from each of the four pens \(\left(\text{corresponding to the four treatments}\right)\) described above and mixed individually using a mortar mixer \(\left(0.34\ m^3\ \text{capacity; Model 125GH9, Crown Construction Equipment}\right)\) to improve uniformity. Average manure water content was 0.63 kg kg\(^{-1}\) in 2010 and 0.69 kg kg\(^{-1}\) in 2011. Manure was surface applied at a rate of ~90 kg wet wt. plot\(^{-1}\), which corresponds to 60 Mg ha\(^{-1}\) \(\left(\text{wet wt.}\right)\), an application rate that is commonly used on irrigated land by farmers in western Canada. Manure was incorporated into the 0- to 10-cm soil layer with a single pass of a disk harrow on 7 and 8 Oct. 2010 and on 11 Oct. 2011. Cross contamination among antimicrobial treatments was prevented by pressure-washing the equipment between treatments as needed. Manure samples were collected from the mixed manure immediately before field application for determination.
of antimicrobial concentrations. The samples were freeze-dried, ground to pass through a 2-mm screen, and maintained at \(-30^\circ C\) until antimicrobials were extracted.

**Soil Sample Collection**

Samples of manure-amended soil were collected from the 0- to 10-cm soil layer using a 5-cm-diameter split core slide hammer. When soil conditions were conducive, a truck-mounted Giddings soil sampler (Giddings Machine Co.) was used during the monitoring period. Four soil cores were randomly collected from each plot and combined to make one composite sample per plot. The initial set of soil samples was taken the day after manure application in each year. During the first 30 d after manure application, soil samples were collected weekly from the TYL treatment (because tylosin has a short DT_{50} in soil) and biweekly from the CTC, CTC-SMZ, and control treatments. Thereafter, samples were collected at \(\sim 30\)-d intervals. Final soil samplings occurred on 16 Aug. 2011 and 14 Aug. 2012 (i.e., \(\sim 10\) mo after manure application). Soil samples were freeze-dried, ground (<2 mm), and maintained at \(-30^\circ C\) until antimicrobials were extracted.

**Antimicrobial Extraction**

**Microwave-Assisted Extraction**

Manure and manure-amended soil samples were subjected to microwave-assisted extraction (MAE) using a Mars Xpress microwave system (CEM Corp.). Freeze-dried and ground manure-amended soil or manure (1 g) was placed in 75-mL Teflon microwave digestion vessels (CEM Corp.). Each sample was extracted with 60 mL of an 80:20 methanol/0.5 mol L\(^{-1}\) citric acid buffer (adjusted to pH 5 with sodium hydroxide) followed by extraction in 40 mL of citric acid buffer. For both extractions, the solvent/sample mixture was heated to 75°C (in 10 min), and this temperature was maintained for 5 min. The two extracts (80:20 methanol/citric acid buffer and citric acid buffer solution) were decanted from the digestion vessel into a single 100-mL beaker and then transferred into two 50-mL polypropylene centrifuge tubes (Fisher Scientific). The extract was centrifuged for 10 min at 3100 rpm (52 Hz), and the supernatant from each centrifuge tube was transferred into a 500-mL amber bottle for overnight storage at 4°C.

**Solid-Phase Extraction and Elution**

The MAE extract was subjected to solid-phase extraction cleanup using a modification of the procedure described by Jacobsen et al. (2004) and Amarakoon et al. (2014) for extracts arising from the pressurized liquid extraction of manure. The MAE extract (\(\sim 100\) mL; extracted the day before as described above) was diluted to 500 mL with milli-Q water to reduce the methanol concentration. An Oasis hydrophilic–lipophilic balance cartridge (225 mg of sorbent, 60 \(\mu\)m particle size; Waters) was stacked on top of an Oasis weak cation exchange cartridge (225 mg of sorbent, 60 \(\mu\)m particle size; Waters), and both cartridges were simultaneously conditioned with methanol (10 mL) followed by milli-Q water (10 mL). The diluted extract (500 mL) was then passed through the solid-phase extraction assembly at a flow rate of 1 mL min\(^{-1}\), followed by rinsing with milli-Q water (10 mL) to remove salts. The cartridges were air-dried under vacuum for 1 min and maintained at 4°C until elution.

The hydrophilic–lipophilic balance cartridge was eluted with methanol (8 mL) into a graduated glass centrifuge tube. The weak cation exchange cartridge was eluted with methanol (4 mL) followed by methanol containing 2% formic acid (4 mL) into a separate graduated glass centrifuge tube. Each eluent was concentrated (500 \(\mu\)L) under a gentle stream of air, followed by dilution with milli-Q water to 1 mL, and transferred into a 2-mL amber liquid chromatography vial through a 0.45-\(\mu\)m nylon membrane syringe filter (Chromatographic Specialties Inc.). Extracts were stored at \(-30^\circ C\) until analysis by liquid chromatography-tandem mass spectrometry (LC/MS/MS). Each eluent was fortified with 100 ng \(^{13}\)C\(_6\)-sulfamethazine (Cambridge Isotope Laboratories) before analysis.

**Liquid Chromatography Tandem Mass Spectrometric Analysis**

All manure and manure-amended sample extracts were analyzed by LC/MS/MS with a Waters 2965 Alliance Separation Module interfaced with a Micromass Quattro Ultima triple quadrupole mass spectrometer (Waters Canada), using operating conditions previously described by Cessna et al. (2011) and Amarakoon et al. (2014). Briefly, a C-18 stainless steel column (100-mm by 2.1-mm i.d., 3.5-\(\mu\)m diam. packing; MS Xterra, Waters Canada) was used for analyte separation. Gradient elution was performed with two mobile phases, both containing acetonitrile/water and 0.1% formic acid. The gradient elution used 90% of mobile phase A (acetonitrile/water, 10:90 v/v) and 10% of mobile phase B (acetonitrile/water, 90:10 v/v) for 1 min followed by 100% of mobile phase B for 9 min. At 10 min, reconditioning of the column for the next injection (at 15 min) was undertaken by switching back to 90% mobile phase A and 10% mobile phase B. The mobile phase flow rate was 200 \(\mu\)L min\(^{-1}\), and the injection volume was 20 \(\mu\)L. Retention times were 6.83 min for tylosin, 6.92 min for iso-chlortetracycline, and 7.11 min for sulfamethazine and \(^{13}\)C\(_6\)-sulfamethazine (internal standard). The electrospray ionization interface was set to positive ion mode. Suitable multiple reaction monitoring transitions were used for confirmation and quantification of antimicrobials (Amarakoon et al., 2014). Data were processed using MassLynx software (v.4.1, Waters Canada), and concentrations were reported on a dry-weight basis.

**Antimicrobial Recovery**

Freeze-dried samples (1 g) of manure and manure-amended soil from their respective control treatments were fortified with 100 \(\mu\)L of a 1 mg L\(^{-1}\) mixture of chlortetracycline, sulfamethazine, and tylosin in Milli-Q water. This fortification solution was prepared by diluting 100-fold a 100 mg L\(^{-1}\) stock solution of each antimicrobial in acetonitrile. Thus, the fortification level was equivalent to 100 \(\mu\)g kg\(^{-1}\) of manure or manure-amended soil. The fortified samples were then extracted and antimicrobial concentrations were quantified as described above.

Average recoveries from manure-amended soil (\(n = 30\)) fortified at 100 \(\mu\)g kg\(^{-1}\) were 66 ± 26% for iso-chlortetracycline, 67 ± 21% for sulfamethazine and 110 ± 43% for tylosin; these values are consistent with those reported for these compounds in agricultural soils (e.g., Jacobsen et al., 2004). Average recoveries from beef cattle manure (\(n = 4\)) fortified at 100 \(\mu\)g kg\(^{-1}\) were 71 ± 24% for iso-chlortetracycline, 24 ± 3% for sulfamethazine,
and 47 ± 9% for tylosin. Simultaneous extraction and analysis of antimicrobials commonly used in livestock from soil and manure is a step forward in enhancing the ability and efficiency of monitoring these compounds in the environment. However, given the different physio-chemical properties of the antimicrobials tested in the present study, the technique compromised the recovery of each antimicrobial.

Average background interferences, most likely resulting from coeluting compounds from control samples of manure-amended soil, were 1.7 μg kg⁻¹ for iso-CTC, 0.13 μg kg⁻¹ for sulfamethazine, and 1.3 μg kg⁻¹ for TYL. For manure samples, the background interferences were ±0.3 μg kg⁻¹. The method detection limit was taken to be three times the background concentration (signal to noise ratio of 3:1), which translates to 5.0 μg kg⁻¹ for manure-amended soil and 1.0 μg kg⁻¹ for manure. The limit of quantification was taken to be 10 times the background concentration (signal to noise ratio of 10:1), which corresponds to 20 μg kg⁻¹ for manure-amended soil and 10 μg kg⁻¹ for manure. Results presented herein are not corrected for recovery. Kinetic parameters were not determined for sulfamethazine and tylosin because their concentrations were below the limit of quantification.

### Statistical Analysis

Dissipation data for chlortetracycline in manured soils were analyzed using PROC NLIN in SAS 9.4 (SAS Institute, 2013) and were best described by the first-order kinetic model:

\[ C_t = C_0 e^{-kt} \]

where \( C_t \) is the antimicrobial concentration (μg kg⁻¹) at time \( t \) (d), \( C_0 \) is the initial antimicrobial concentration (μg kg⁻¹), and \( k \) is the first-order dissipation rate constant (d⁻¹). Dissipation kinetics was evaluated separately for each replicate (block) in each season in each year. Dissipation rate constants estimated by the NLIN procedure for chlortetracycline and the corresponding DT₅₀ (0.693/k) data followed a lognormal distribution and were subjected to ANOVA using PROC GLIMMIX in SAS with treatment (CTC and CTCSMZ) and season (nongrowing [1 October–30 March] and growing [1 April–31 August]) as fixed effects and block and year as random effects. The Tukey multiple comparison procedure was used for pairwise comparison of treatment means if a significant effect was indicated by the global ANOVA. Treatment differences were considered significant at \( P < 0.05 \).

Dissipation kinetics for sulfamethazine and tylosin were not assessed because concentrations of these antimicrobials in manure-amended soil were low. Mean, maximum, and minimum soil and air temperatures were determined with PROC MEANS in SAS.

### Results and Discussion

#### Temperature and Precipitation

Daily mean soil temperature at the 5-cm depth ranged from −5 to 23°C (mean, 6.5°C) in 2010–2011 and from −3.2 to 27°C (mean, 8.0°C) in 2011–2012 (Fig. 1). The soil temperatures during the study period were ≤0°C for 108 d in 2010–2011 and for 81 d in 2011–2012. Because soil temperatures ≤0°C occurred mainly in the nongrowing season months of November through February, the upper soil layer into which manure was incorporated would have been frozen for much of this period. Daily mean air temperature during the study ranged from −25

#### Antimicrobial Concentrations in Manure

Manure accumulated in the feedlot pens for 111 d in 2010 and for 105 d in 2011. The period of manure accumulation was drier in 2011 (105 mm of rain) than in 2010 (138 mm). Concentrations of all three antimicrobials tended to be higher in 2011 (Table 1).

Mean antimicrobial concentrations in manure decreased in the order chlortetracycline > sulfamethazine > tylosin (Table 1). The concentration of chlortetracycline in manure did not differ significantly (\( P > 0.05 \)) between CTC and CTCSMZ in each year of manure application. Sulfamethazine concentration was more than an order of magnitude (13- to 20-fold) lower than that of chlortetracycline in manure from CTCSMZ even though it was administered at the same concentration in feed. These relative antimicrobial concentrations are consistent with those
observed by Cessna et al. (2011) in beef cattle feedlot manure but are in contrast to the higher excretion rates reported by Kim et al. (2011) for sulfamethazine (~90%) than for chlortetracycline (~65–75%). The lower concentration of sulfamethazine in the manure in the present study may reflect its greater mobility than chlortetracycline (Chee-Sanford et al., 2009); thus, some sulfamethazine may have been transported off-site in rainfall runoff during the period of manure accumulation in feedlot pens. Rainfall amounts capable of producing surface runoff from the feedlot pens (Miller et al., 2004; Olson et al., 2006) were recorded in 2010 (13.6 and 24.5 mm) and 2011 (15.4, 15.6, and 37.0 mm). A fraction of sulfamethazine may have converted to its metabolite, N-acetyl-sulfamethazine (Haller et al., 2002), which was not analyzed for in the present study; this likely reduced the sulfamethazine concentration detected in manure. Sulfamethazine forms strong hydrogen (Teixido et al., 2011) and covalent (Bialk et al., 2005; Gulkowska et al., 2013) bonding with manure, and the bonding gets stronger with extended contact time (Carstens et al., 2013; Stoob et al., 2006, 2007; Yang et al., 2009), such as occurred in this study during manure accumulation in the feedlot; this may also have reduced the sulfamethazine concentration measured in manure. Tylosin was administered at a lower concentration in the feed than chlortetracycline and sulfamethazine, resulting in its lower initial concentration in manure. Further, rapid dissipation of tylosin in manure has previously been reported (De Liguoro et al., 2003; Hamscher et al., 2002; Kay et al., 2004).

**Initial Antimicrobial Concentrations in Manure-Amended Soil**

Antimicrobial concentrations in manure-amended soil generally reflected the antimicrobial concentrations in the applied manure (Table 1) and decreased in the order chlortetracycline > sulfamethazine > tylosin. However, the high standard deviations (±20 to ±96%) among initial samples is reflective of the fact that manure was not homogeneously distributed throughout the 0- to 10-cm soil layer after incorporation. In addition, the initial concentration of chlortetracycline in the manure-amended soil relative to that in the applied manure from both CTC and CTCSMZ showed that incorporation into soil diluted the concentration of chlortetracycline by approximately two orders of magnitude. A similar dilution was observed for sulfamethazine and tylosin. The maximum concentrations of chlortetracycline in the manure-amended soil from CTC (140 μg kg⁻¹) and CTCSMZ (210 μg kg⁻¹) occurred in 2011. Maximum initial concentrations of 16 μg kg⁻¹ for sulfamethazine and 11 μg kg⁻¹ for tylosin were also measured in manure-amended soil in 2011 when corresponding concentrations in the manure tended to be higher.

**Dissipation Kinetics for Chlortetracycline**

Dissipation of chlortetracycline during the study period after manure application was best described by the first-order kinetic model. Similarly, previous studies have demonstrated first-order kinetics for chlortetracycline in soil (Carlson and Mabury, 2006; Halling-Sørensen et al., 2005) and during composting of beef cattle manure (Cessna et al., 2011). There was a significant (P = 0.04) treatment by season (growing vs. nongrowing season) interaction for the DT₅₀ for chlortetracycline (Fig. 3). Chlortetracycline from the CTCSMZ treatment dissipated significantly faster (P = 0.03) during the growing season (DT₅₀ = 77 d) than during the nongrowing season (DT₅₀ = 648 d), which may suggest that the dominant dissipation pathway involved may depend on temperature. By comparison, dissipation of chlortetracycline from the CTC treatment did not differ significantly between the two seasons (mean DT₅₀ = 121 d), suggesting a different dissipation pathway relative to the CTCSMZ treatment. During the nongrowing season, chlortetracycline from the CTC treatment dissipated faster (P = 0.004) than that from the CTCSMZ treatment, whereas the DT₅₀ values did not differ significantly between CTC and CTCSMZ during the growing season. This result indicates that during the nongrowing season chlortetracycline in manure-amended soil dissipates faster when applied alone (CTC) than when applied as a 1:1 mixture of chlortetracycline and sulfamethazine (CTCSMZ). This suggests that the presence of sulfamethazine may have altered (i.e., slowed down) the dissipation of chlortetracycline during the nongrowing season. This is the first time that a study has shown that the presence of sulfamethazine in the medium reduces the dissipation of chlortetracycline. It is not clear, however, why this effect was not evident in the growing season, but this may be due to the higher microbial activity during the growing season. As noted earlier, all previous field studies that we are aware of were conducted during the growing season.

To our knowledge, this study is the first to examine the dissipation of the three antimicrobials over a lengthy period (10 mo).

### Table 1. Antimicrobial concentrations (dry wt. basis) in manure and soil.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Treatment†</th>
<th>Year of application</th>
<th>Antimicrobial concentration‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Manure</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>CTC</td>
<td>2010</td>
<td>9,180 (1,610)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2011</td>
<td>15,700 (960)</td>
</tr>
<tr>
<td></td>
<td>CTC</td>
<td>2010</td>
<td>6,970 (1,330)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2011</td>
<td>13,400 (1,400)</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>CTCSMZ</td>
<td>2010</td>
<td>520 (110)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2011</td>
<td>600 (64)</td>
</tr>
<tr>
<td>Tylosin</td>
<td>TYL</td>
<td>2010</td>
<td>210 (51)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2011</td>
<td>230 (49)</td>
</tr>
</tbody>
</table>

† CTC, chlortetracycline administered alone in the animal feed; CTCSMZ, a 1:1 mixture of chlortetracycline and sulfamethazine; TYL, tylosin.
‡ Antimicrobial concentrations in manure (on day of manure application) and in soil (1 d after manure application). Concentrations are arithmetic means of four replicates; values in parentheses are standard deviations. ND, not detected.
that included winter months, during which soils were frozen for up to 108 d. The DT 50 values estimated for chlortetracycline during the growing season are in general agreement with results from similar field studies conducted elsewhere during warm months (Carlson and Mabury, 2006; Halling-Sørensen et al., 2005). The greater persistence we observed for chlortetracycline from the CTCSMZ treatment during the nongrowing season relative to the growing season reflects low mean annual temperatures in this seasonally frozen soil. However, it is not clear why the seasonal effect was not significant for the chlortetracycline from the CTC treatment.

Temperature dependency of chlortetracycline dissipation has previously been reported in a 30-d laboratory incubation study (Gavalchin and Katz, 1994) in which 56% of the added chlortetracycline was dissipated at 30°C compared with just 12% at 20°C and no significant dissipation at 4°C. Also, Loftin et al. (2008) showed that the DT 50 of chlortetracycline in soil increased as temperature decreased from 35°C through 22 to 7°C. Chlortetracycline has soil sorption coefficients (K d) of 1208 to 2386 L kg$^{-1}$ (Sarmah et al., 2006), and because it has three acid dissociation constants (pK a1 = 3.3, pK a2 = 7.4, and pK a3 = 9.3), it can exist as a cation (+0+), a zwitterion (+–0), and/or an anion (+––). Consequently, cation exchange would be the main mechanism of chlortetracycline sorption in soils with high cation exchange capacities (Pils and Laird, 2007; Sarmah et al., 2006; Sassman and Lee, 2005), such as those on the Canadian prairies (Soil Classification Working Group, 1998). Although the sorption of chlortetracycline to soil tends to decrease with increasing pH (Essington et al., 2010), a significant fraction of chlortetracycline remains sorbed at environmentally relevant pH values (Figueroa-Diva et al., 2010). Thus, the persistence of chlortetracycline observed in the present study was likely due in part to its strong sorption to soil components postapplication in the soil, thus making it less available for microbial degradation.

Sulfamethazine and Tylosin

Because the concentrations of sulfamethazine and tylosin in soil were low throughout the study periods with no apparent trends over time (Fig. 4), it was not possible to determine their dissipation kinetics. However, both sulfamethazine and tylosin were detected in the manure-amended soil in the month of manure incorporation in each year, and sulfamethazine was continually detected in soil at low concentrations during both 10-mo monitoring periods. Tylosin concentration in soil was also low, but, unlike sulfamethazine, tylosin was gradually dissipated.

Conclusions

Antimicrobial concentrations in manure-amended soil generally reflected antimicrobial concentrations in the applied manure and decreased in the order chlortetracycline > sulfamethazine > tylosin. The first-order dissipation half-life of chlortetracycline when added as a 1:1 mixture with sulfamethazine was 77 d during the growing season and 648 d during the nongrowing season when topsoil was frozen for an extended period. By comparison, dissipation of chlortetracycline from the CTC
treatment did not differ significantly between the two seasons (DT$_{50}$ 121 d). Both sulfamethazine and tylosin were detected at low concentrations in manure-amended soil; sulfamethazine was detected throughout the monitoring period, whereas tylosin was gradually dissipated. Chlortetracycline had greater persistence than reported in previous studies, reflecting the slow dissipation in the seasonally frozen soil. Chlortetracycline was measured in the soil 10 mo after manure application, indicating a potential risk for residue build-up in the soil and for off-site contamination if manure is applied repeatedly to the same field.

Acknowledgments

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